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INVESTIGATING THE PHYTOCHEMICAL PRODUCTION AND ANTIOXIDANT ACTIVITY IN FOUR PLANT SPECIES BELONGING TO THE *ASTERACEAE* FAMILY

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ABSTRACT

Vietnam has a rich and profound traditional medicine system that is widely used today. Medicinal plants are used to treat colds, coughs, bone and joint diseases, digestion, respiratory diseases, *etc*. In this study, leaves of *Artemisia vulgaris, Taraxacum officinale, Blumea balsamifera,* and flowers of *Xerochrysum bracteatum* from the *Asteraceae* family were selected to determine the antioxidant capacity and relationship with the active ingredients in the plants. The methanolic extracts were screened for chemical compositions via the total phenolic content (TPC) assay, the total flavonoid content (TFC) assay, and DPPH radical scavenging activity. The highest radical scavenging activity was observed in the flowers of *X. bracteatum* (IC_{50} = 0.061 mg/mL), followed by the leaves of *Taraxacum* (IC_{50} = 0.313 mg/mL), *A. vulgaris* (IC_{50} = 1.367 mg/mL), and *B. balsamifera* (IC_{50} = 1.4502 mg/mL). The TPC of the studied plants ranged from 19.98 ± 1.355 to 195.78 ± 42.518 mgGAE/g extract, while the TFC ranged from 60.31 ± 1.725 to 339.14 ± 26.299 mgQE/g extract. The highest TPC and TFC were recorded in the methanol extract of *X. bracteatum*. The strongly negative correlation between the TPC and TFC and the IC_{50} values (R^2 = -0.78, R^2 = -0.76) suggests that TFC and TPC could strongly contribute to the antioxidant activity of these plants. These results not only highlight the relevance of these plants in traditional medicine but also scientifically validate their use, particularly in the context of their antioxidant properties. The study underscores the close relationship between the traditional use of these plants and their scientifically observed effects, reinforcing the value of folk remedies.

Keywords: Antioxidant, *Asteraceae*, flavonoid, free radical scavenging activity, phenolic, secondary metabolites

INTRODUCTION

The *Asteraceae* family, commonly called the sunflower family, is a remarkably diverse and widespread group of flowering plants comprising over 1.600 genera and approximately 25.000 species distributed across the globe, except Antarctica. It

includes many well-known plants, such as chicory, sunflower, daisies, and dandelion, which are renowned for their potential medicinal functions, including antioxidant, antimicrobial, anticancer, and antidiabetic (Rolnik & Beata, 2021).

Furthermore, the *Asteraceae* family is characterized by a rich diversity of phytochemical compounds, including polyphenols, phenolic acids, flavonoids, sesquiterpene lactones, essential oils, saponins, and lignans (Rolnik *et al*., 2021). This complex chemical composition contributes to the biological activities exhibited by many species within the family, making them valuable resources in traditional medicine practices across various cultures.

Artemisia vulgaris, or mugwort, is a perennial herb with a fragrant and bitter taste. It has unique pharmacological properties that are used in traditional medicinal systems of China, Europe, India, and Vietnam. According to traditional medicine, mugwort is used as a medicine and food with the therapeutic effect of warming the meridians, relieving pain, dispelling dampness, and dispersing cold, so it is used to treat diseases such as convulsions, fever, malaria, regulating menstruation, and the digestive tract (Thangjam *et al*., 2020) thanks to its important phytochemicals such as oil, phenol, flavonoids, terpenoids, and saponin, as well as tannin (Thangjam *et al*., 2020; Ashok & Upadhyaya, 2013).

Blumea balsamifera (sembung plant) is a traditional medicine widely used across Asian cultures for its diverse health benefits. In traditional systems, every part of the *B. balsamifera* plant has been employed to treat numerous ailments, such as remedies for coughs, colds, fever, and joint pain, even relieving skin infections and inflammation (Pang *et al*., 2014). Its various pharmacological actions are supported by studies demonstrating high antioxidant activity with powerful bioactive compounds such as flavonoids and phenolic acids (Dai *et al*., 2023). Additional investigations have shown it possesses anti-inflammatory, antibacterial, and hepatoprotective effects (Widhiantara & Jawi, 2021).

Xerochrysum bracteatum, commonly known as golden everlasting and *Helichrysum bracteatum* for many years before, is an aromatic herbaceous plant native to eastern Australia. *Helichrysum* species have been recognized for their therapeutic aspects as diuretic, anti-inflammatory, hepatoprotective, anti-psoriasis, antimicrobial, and antioxidant activities (Najar *et al*., 2019; Akinyede *et al*., 2021).

Taraxacum officinale, known as dandelion, is a recognizable worldwide herb in temperate grasslands and pastures. Dandelion originated from Europe and has a long history of use in folk medicine across different traditional systems, treating liver and digestive problems (Fan *et al*., 2023; Schütz *et al*., 2006). Many authors reported the antioxidant, anti-inflammatory, anti-cancer, and other effects of dandelion (Jalili *et al*., 2020) thanks to its phenolics, flavonoids, terpenes, and coumarins (Yan *et al*., 2024).

This study aimed to determine the antioxidant potential and free radical scavenging activity of wild medicinal plant species belonging to the *Asteraceae* family. A range of assays would be used to evaluate each plant species' antioxidant capacity and ability to scavenge free radicals. Additionally, the total phenolic and flavonoid content would be quantified and

correlated with the observed antioxidant activity to analyze their relationship. By providing scientific evidence of their antioxidant profiles and phytochemical contents, the study intended to validate the traditional uses of the plant species.

MATERIALS AND METHODS

Plants materials

Mature leaves of *Artemisia vulgaris, Taraxacum, Blumea balsamifera,* and flowers of *Xerochrysum bracteatum* of the 2-year-old plants from the *Asteraceae* family were collected in Ninh Binh province and dried for further studies.

Methods

Preparation of methanolic extracts

The samples were cleaned and dried at 60^{o}C until they reached a constant weight. Once dried, they were ground into a fine powder and placed in separate vials. Our study used methanol as the solvent to extract the bioactive compound from the samples. One gram of every sample added with 99.5% methanol (Xilong) at the 1:11 (v/v) ratio was sonicated at 38^{o}C for 30 minutes, followed by being centrifuged at 13000 rpm 4^{o}C for 10 minutes. The supernatant, containing the crude extract, was then evaporated in an oven set at 60^{o}C for 2 days to remove the methanol and concentrate the bioactive compounds. The extract mass was determined and the concentration was standardized by adding a certain amount of methanol for the final 40 mg/mL concentration.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

In vitro antioxidant activities of the extracts were determined using the DPPH free radical scavenging assay, described by Mai *et al*. (2024). The ascorbic acid was used as a positive control. In each reaction, 40 µL of the plant extract was mixed with 760 µL of DPPH 0.25 mM (Sigma-Aldrich) and incubated at room temperature for 20 minutes in the dark. The absorbance of the mixture was measured at 517 nm by UV-Vis spectrophotometer (SpectraMax® iD5) against the blank solution.

Total phenolic content (TPC)

The TPC in the methanolic extracts was estimated using the Folin-Ciocalteu colorimetric method (Singleton *et al*., 1999) with some modifications. Gallic acid (GAE) (Acros Organics) was prepared for the standard calibration curve. In each sample, 40 µL of the plant extract was mixed with 480 µL of Folin-Ciocalteu reagent 10% (Merck) and then incubated at 40^{o}C for a minute. Thus, 480 µL of sodium carbonate 6% was added and continuously incubated at 40^{o}C for 15 minutes. The absorbance of the blue-colored mixtures was measured at 765 nm by using the UV-Vis spectrophotometer (SpectraMax® iD5) against the blank solution. The TPC value was expressed as mg GAE/g extract.

Total flavonoid content (TFC)

The TFC in the methanolic plant extracts was estimated using the aluminum chloride colorimetric method (Phuyal *et al.*, 2020) with slight modification. Quercetin (QE) (Aldrich Sigma) was prepared for the standard calibration curve. In each reaction, 240 µL of plant extract was mixed with 40 µL of 5% sodium nitrite solution and incubated at 27^{o}C for 6 minutes. After

incubation, 40 µL of 10% aluminum chloride solution was added to the mixture and continuously incubated for 6 minutes. Thus, 400 µL of NaOH 1M and 280 µL of ethanol 30% were added simultaneously and left for 30 minutes. Finally, the OD of mixtures was measured at 510 nm by UV-Vis spectrophotometer (SpectraMax® iD5) against the blank solution. The TPC value was expressed as mg QE/g extract.

Statistical Analysis

All the experiments were repeated three times. The data was presented by means of three replicates ± standard deviation. The difference between parameters was analyzed using one-way ANOVA and Turkey's post-hoc test to assess the statistical significance of the means, with $p < 0.05$ considered significant.

RESULTS

Total phenolic contents in four studied plants

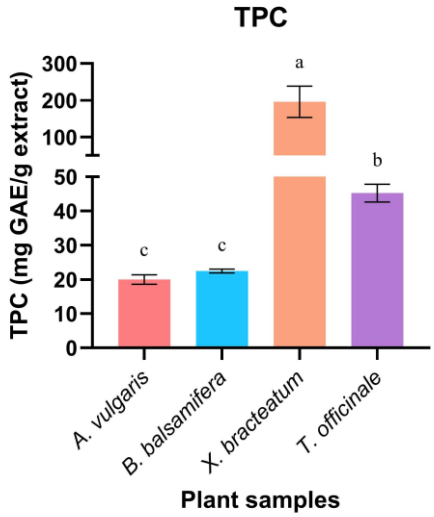

Figure 1. Total phenolic contents in four studied plants from the *Asteraceae* family

The analysis of TPC presented in Figure 1 highlighted significant variations among four plant species in the *Asteraceae* family - *A. vulgaris*, *B. balsamifera*, *X. bracteatum*, and *T. officinale*. An exceptionally high TPC of 195.78 ± 42.52 mg GAE/g was demonstrated by *X. bracteatum*. A moderate TPC of 45.22 ± 2.58 mg GAE/g ($p < 0.05$) was shown by *T. officinale*. In contrast, a lower TPC of 22.45 ± 0.56 mg GAE/g was recorded for *B. balsamifera*, which was not significantly different ($p > 0.05$) from *A. vulgaris*, the species with the lowest TPC at 19.98 ± 1.35 mg GAE/g. These findings suggested that *X. bracteatum* was likely the most potent source of phenolic compounds among the species studied, making it a valuable candidate for applications requiring high antioxidant capacity.

Total flavonoid content in four studied plants

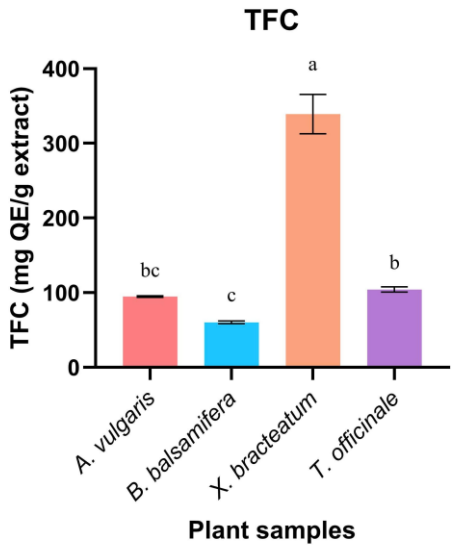

Figure 2. Total flavonoid content in four studied plants from the *Asteraceae* family

Based on the TFC data shown in Figure 2 for the four plant species, significant differences were revealed among them. The highest TFC value of 339.145 ± 26.299 mg QE/g was exhibited by *X. bracteatum*, which was found to be substantially higher than that of the other species ($p<0.05$), indicating a strong presence of flavonoids. Conversely, the lowest TFC was observed in *B. balsamifera*, with a mean of 60.309 ± 1.725 mg QE/g ($p<0.05$). Intermediate TFC values were recorded for *A. vulgaris* and *T. officinale*, with means of 94.813 ± 0.993 mg QE/g and 104.321 ± 3.443 mg QE/g extract, respectively. The TFC of *A. vulgaris* was found to be significantly different from that of *B. balsamifera* but not from *Taraxacum*. While the TFC of *T. officinale* was determined to be statistically higher than that of *B. balsamifera* but lower than *X. bracteatum*.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

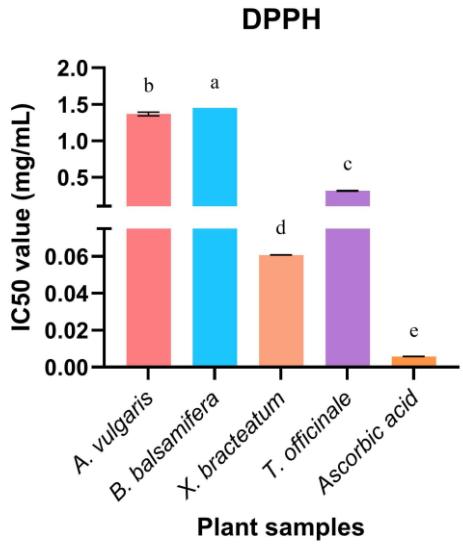

Figure 3. IC_{50} values of four samples for free radical scavenging activity by DPPH radical.

Figure 3 shows the DPPH radical scavenging activity, measured as IC_{50} values, for four plant species within the *Asteraceae* family, along with ascorbic acid as a reference standard. Ascorbic acid exhibited the lowest IC_{50} value of approximately 0.006 mg/mL. Among the plant species tested, although it is significantly ten times less potent than ascorbic acid, *X. bracteatum* demonstrated the strongest antioxidant activity with an IC_{50} value of approximately 0.061 mg/mL. *T. officinale* also exhibited moderate antioxidant activity, with an IC_{50} of approximately 0.313 mg/mL. In contrast, *A. vulgaris* and *B. balsamifera* showed weaker antioxidant activities, with IC_{50} values of approximately 1.367 mg/mL and 1.45 mg/mL, respectively.

The correlation between antioxidant parameters in four studied plants from the Asteraceae family

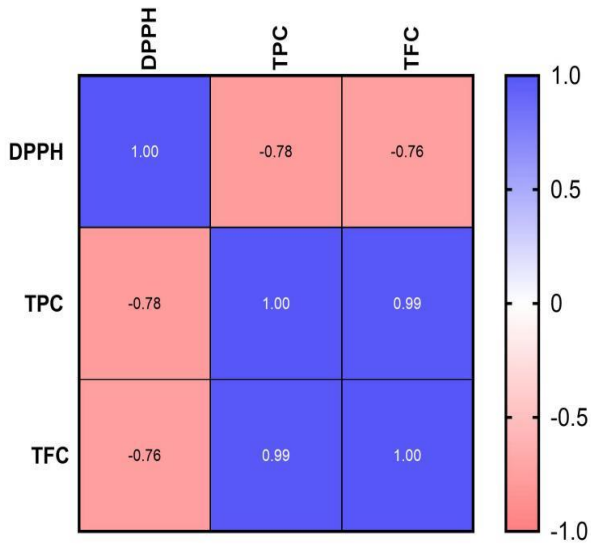

Figure 4. Pearson correlation matrix between DPPH, TPC, and TFC in four extracts from studied plants in the *Asteraceae* family.

The Pearson correlation matrix presented in Figure 4 highlighted the relationships between DPPH radical scavenging activity, TPC, and TFC for the four plant species belonging to the *Asteraceae* family that were studied. A strong negative correlation was observed between DPPH and TPC, with a correlation coefficient of -0.78. This suggested that higher total phenolic content was associated with stronger antioxidant activity, as indicated by lower DPPH IC_{50} values. Similarly, a strong negative correlation of -0.76 was found between DPPH and TFC, indicating that higher flavonoid content was also linked to increased antioxidant activity. An almost perfect positive correlation of 0.99 was observed between TPC and TFC, suggesting that in these plants, phenolic and flavonoid contents were closely related, likely because flavonoids are a subclass of phenolic compounds. These findings implied that the plants with higher TPC and TFC, such as *X. bracteatum*, were likely to exhibit stronger antioxidant properties.

DISCUSSION

This study investigated the antioxidant properties and phytochemical composition of some medicinal plants of the *Asteraceae* family commonly used in traditional medicine in various cultures, especially in Vietnam. Specifically, we evaluated *Artemisia vulgaris*, *Blumea balsamifera*, *Xerochrysum bracteatum*, and *T. officinale* species to determine their total phenolic, flavonoid content, and free radical scavenging capacity.

Our study on *A. vulgaris* showed lower TPC, TFC, and weaker antioxidant activity compared to other *Asteraceae* species, with TPC of 19.98 ± 1.35 mg GAE/g, TFC of 94.81 ± 0.99 mg QE/g and IC_{50} value of 1.367 mg/mL in the DPPH assay. Differently, the study by Sharma *et al.*

(2023), which also used methanol as solvent to extract metabolites, found higher TPC and antioxidant activity but lower TFC in *A. vulgaris* grown in Nepal, with TPC of 26.04 ± 1.66 mg GAE/g, TFC of 33.41 ± 0.27 mg QE/g and IC_{50} of 149.62 ± 2.40 µg/mL for leaf extract from Chitwan, Nepal. These differences highlight the significant impact of environmental factors, such as geographical location (Ninh Binh, Vietnam vs. Chitwan, Nepal), as well as differences in extraction methods and experimental conditions on the phytochemical composition and biological activities of *A. vulgaris*. Although our study observed lower TPC and antioxidant activity but higher TFC than Nepalese samples, *A. vulgaris* still exhibited valuable bioactive properties. This suggests that with further optimization of extraction techniques and exploration of different growth conditions, the antioxidant potential and therapeutic applications of *A. vulgaris* could be significantly enhanced.

Although in our report, *B. balsamifera* exhibited lower antioxidant activity than other species in the *Asteraceae* family, they are still regarded as having the potential for exploitation due to their numerous pharmacological benefits, including pain relief and fever reduction, *etc*. Zulkiflee. (2022) conducted a study on the TPC and TFC of *B. balsamifera* using a variety of extract solvents, including ethanol, methanol, and hexane, which the TPC and TFC contents of *B. balsamifera* extract with methanol were approximately four times lower than the results stated in our report, even with other extract solvents. On the other hand, in the report of Rawati *et al.* (2023), the hexane-extracted *B. balsamifera* sample demonstrated a lower IC_{50} value than the methanol-extracted sample in our report. This indicates that the hexane-extracted sample had a superior DPPH radical scavenging ability. The discrepancy may be attributed to the different extraction methods, sample extraction solvents, or growth media. Besides, the scavenging capacity of a sample in the DPPH radical scavenging experiment is determined by its electron or hydrogen atom transfer potential, which is controlled by the redox characteristics of the phytochemicals present in the sample. While flavonoids and phenolic compounds are frequently efficient DPPH radical scavengers, their efficacy can differ depending on their particular chemical compositions and the existence of other substances that may influence their action.

Out of the species investigated from the *Asteraceae* family, *X. bracteatum* was identified as the most effective antioxidant plant. Compared to research by Kandylis, 2022, the overall finding indicated that *X. bracteatum* exhibited a superior capacity to scavenge free radicals compared to the other plant species. In addition, our findings indicated a notably greater phytochemical content in Vietnamese *X. bracteatum* compared to Kandylis' research. Particularly, this was proven through the phenolic and flavonoid content analysis, revealing that the Vietnamese *X. bracteatum* flowers have approximately triple the number of phenolic compounds. The differences can be explained by the differences in environmental conditions such as soil, and climate. Although *X. bracteatum* is well-known for its antibacterial and anti-inflammatory properties, this discovery has highlighted its important potential in combating free radicals.

Compared to other plant extracts in the *Asteraceae* family, the methanol extract of Vietnamese *T. officinale* exhibited moderate antioxidant properties, with an IC_{50} of

approximately 0.313 mg/mL, which was comparable to that reported for *T. officinale* grown in Turkey, which showed an IC_{50} of 0.482 mg/mL (Ergün, 2021). Furthermore, the high DPPH scavenging ability exhibited by the Vietnamese extract corroborates the traditional medicinal use of this species to treat diseases associated with oxidative stress and free radical-induced tissue damage. Besides, the quantitative phytochemical analysis revealed the extract contained significant amounts of phenolic compounds (45.225 mg GAE/g) and flavonoids (104.321 mg QE/g) in our study, demonstrating the rich polyphenolic profile of the Vietnamese *T. officinale* leaf extract. Ergün (2021) evaluated 36.53 mg GAE/g and 43.31 mg QE/g in *T. officinale* leaf extract, markedly lower than the present findings. However, Liu *et al*. (2020) extracted the *T. officinale* by fermentation pre-treatment and measured elevated flavonoid levels ranging from 109.49 to 183.72 mg/g, suggesting fermentation optimization of bioactive compounds' yields. This suggested that fermentation processing can improve phenolic and flavonoid biosynthesis and extraction. Notably, the Vietnamese methanol extract of *T. officinale'*s leaf contained flavonoid levels on par with the samples reported by Liu *et al.* (2020) implying the specific regional factors modulate the production of these secondary metabolites.

CONCLUSION

Our study reveals the high potential antioxidant activity of four different plant species belonging to the *Asteraceae* family. Among them, *Xerochrysum bracteatum* appears to be the best candidate for therapeutic application in terms of antioxidant activity, where we obtained the highest TPC, TFC, and strongest DPPH scavenging activity. Further studies need to be conducted to optimize the metabolite extraction process in order to obtain the higher antioxidant compounds or to study the effect of environmental conditions in the production of metabolites.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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